FEEDING AND OVIPOSITION BEHAVIOR IN THE MOSQUITO AEDES AEGYPTI (L.). I. PRELIMINARY STUDIES OF PHYSIOLOGICAL CONTROL MECHANISMS

CHARLES L. JUDSON

Department of Entomology, University of California, Davis, California 95616

Fundamental behavioral patterns associated with feeding and egg-laying have long attracted the attention of biologists, although among insects little is known of the physiological mechanisms controlling these activities. Mosquitoes offer a particularly advantageous organism in which to study these processes, since feeding and oviposition are well-defined endpoints, and because a considerable fund of knowledge on the relation between feeding and ovarian development exists.

The cyclic nature of biting and oviposition by females of *Aedes aegypti* has been recognized for some time (MacFie, 1915; MacGregor, 1931). Lavoipierre (1958), however, was the first to point out the presence of two distinct biting patterns in this species, and demonstrated that these patterns were determined by whether the female was virgin or had mated. A second behavior pattern, again depending on whether or not insemination had occurred, is noted when oviposition is studied. Virgin females, while maturing the same number of eggs following a blood meal as do mated females, retain all, or most, of the eggs. These eggs will be deposited following mating, but in the absence of mating will be retained until the death of the female. Mated females, on the other hand, oviposit readily and completely on completion of oogenesis, provided a suitable site is available. This phenomenon was first reported by both MacFie and MacGregor. Further documentation was provided by Gillett (1955), Lang (1956), Wallis and Lang (1956) and Lavoipierre (1958). In contrast, Gillett also reported a single West African strain of this species in which no difference exists in the oviposition behavior of mated and virgin females, both laying their eggs on maturation.

With this single exception, it is well documented that differences in behavior between mated and virgin females of *A. aegypti* do exist, and that the virgin patterns can be changed to the mated type by some aspect of the mating act. A "system" thus exists which is well suited for investigating the physiological mechanisms by which these patterns can be altered. This paper reports the preliminary findings of this investigation.

METHODS

Mosquitoes of the Liverpool strain of *A. aegypti* which had been cultured in this laboratory for several years were used in this study. To reduce variability, a standard rearing procedure was adapted. Two hundred fifty newly hatched larvae were placed in 500 ml. of water in an $11'' \times 7'' \times 2''$ deep enamel pan. Finely ground cattle food (Misco Mills High Protein Supplement No. 2) was provided as food, 0.60 g. being added per pan on days 2 and 5 (day of hatching equals day 1),

and 1.20 g. on day 7. Preliminary tests showed this to be an excess amount of food for this number of larvae, so that the carryover of nutrients into the adult stage was equalized between batches of mosquitoes.

Pupae were sexed and then isolated in small cages. On emergence adult females were randomly collected and transferred to foot-square plywood and Plexiglas (top and one side) cages. Water and a cube of sucrose were continuously available. When mated females were required, a surplus of males was added to the cage, left for 24 hours, and the males removed at least 24 hours prior to the beginning of feeding tests. Preliminary experiments consisting of examination of ovaries for the presence of mature eggs and the spermathecae demonstrated that all females were inseminated under these conditions.

Females were allowed to engorge on a human hand; non-feeders were removed from the cage so that the population used in these experiments consisted wholly of females which fed during the first opportunity. On subsequent days a 10-minute feeding opportunity was offered this group, and the numbers ingesting blood recorded. Feedings tests were conducted between 9:00 and 10:00 AM each day, to avoid any variation due to a feeding rhythm even though none has been demonstrated in this strain (Khan *et al.*, 1965), and the females always allowed to feed to repletion. Oviposition behavior was determined by continuously providing a small glass or waxed-paper dish containing distilled water and a strip of paper towelling. Egg counts were made daily following the feeding tests.

The possibility that stimuli arising from physical union during copulation were responsible for inducing the mated type of behavior was tested by allowing virgin females to copulate with "depleted" males in the manner of Gillett (1955). These males had previously been allowed to copulate in a separate cage with a minimum of 6 females in rapid succession, and had generally depleted their supply of semen. The test females were introduced singly into a cage containing a few "depleted" males, and were removed immediately following a single union. Microscopic examination of the spermathecae following the tests verified that few or no sperm had been transferred during these matings.

The semen transferred during copulation constitutes another factor which might have been responsible for the "mated effect." Any effects of the components of this fluid were determined by introducing an isolated testis or lobe of the symmetrically bilobed accessory gland into newly emerged virgin females and determining the resulting patterns of biting and oviposition. Females were immobilized by chilling and placed in a restraining device (Lavoipierre and Judson, 1965). The organ under investigation was taken up in a saline-filled micro-pipette and injected into the thorax through a slit in the metapleuron.

The possible participation of the ovaries in the regulation of biting behavior was investigated by implanting a normal ovary into females in which development of these organs had been inhibited. The chemosterilant Apholate (Olin Mathieson Chemical Corp.), added to larval pans (day 2) at a rate of 25–30 ppm interfered with development of the ovary. Only a rudimentary non-functional organ was present in emerging females. An ovary from a normal untreated female was injected into the thorax of these females, and then the female was mated or received an implant when required, and the biting pattern determined. All implants and injections were made using the saline of Hayes (1953), and the females allowed a 36–48-hour recovery period prior to beginning the feeding tests. Feeding or
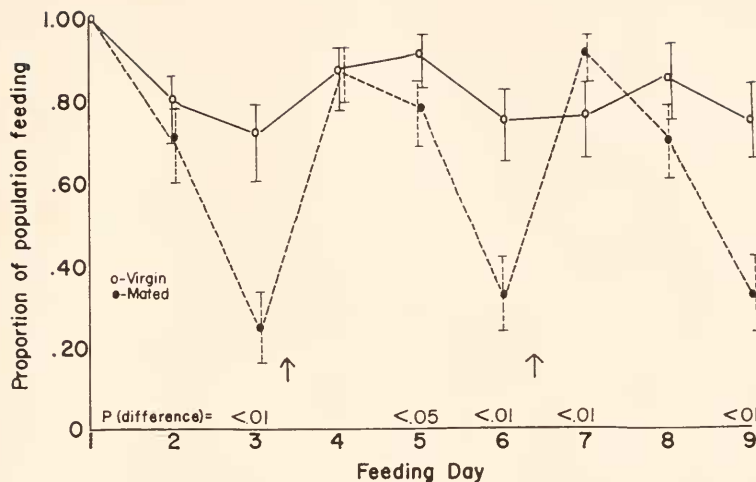
FIGURE 1. Feeding patterns of mated and virgin female *Aedes aegypti,* 95% confidence limits. Arrows denote oviposition by mated females.

biting as used here refers to the actual ingestion of blood. Careful observation during these tests demonstrated that females of this strain did not probe or penetrate the host's skin without ingesting blood, and in fact, any female which was "activated" (Laarman, 1958) to leave the cage wall and alight on the host invariably took blood. The amount of blood ingested varied due to the presence of residues of previous meals in the gut, as well as with the presence or absence of eggs in the ovaries, although some definite expansion of the abdomen always occurred. All rearings and tests were carried out at $80 \pm 4°$ F. The relative humidity of the rearing room was $75 \pm 5\%$, but within the cages was somewhat higher.

RESULTS

The pronounced alteration of the feeding pattern of virgin *A. aegypti* which is induced by mating can be seen in Figure 1. While at least 75% of the virgin female population fed daily, the proportion of mated females feeding on days 3, 6, and 9 was significantly reduced ($p < .01$). These periods of low feeding avidity occur during the terminal portions of the first, second, and third gonotrophic cycles, respectively. Oviposition by mated females (see below) occurs within hours (6–18) of these low points; on the following day eggs had been laid, avidity for a blood meal had increased markedly, and the subsequent gonotrophic cycle initiated. The feeding behavior of virgin females is also cyclic, but weakly so [significantly more females fed on days 1 and 5 than on day 3 ($p = .05$)]. This cycle shows a 4- to 5-day period rather than the precise 4-day period of the mated females.

Since these results clearly indicate that some aspect of the mating act is responsible for modifying the virgin biting and oviposition patterns, tests were conducted to identify the factor(s) responsible, using the proportion of the test population feeding on day 3 or the total number of bites over a 3-day period, as

TABLE I

The effect of repeated matings by males on semen depletion and biting and oviposition by female Aedes aegypti

	No. of copulations by male			Control	
	1–3	4–6	7 plus	Virgin	Mated
No. females/group	21	16	26	12	17
Total bites, days 1–3	41	36	60	31	28
Average bites/female, 3 days	1.9	2.3	2.3	2.6	1.6
Total eggs laid, days 4–6	813	512	234	106	967
Average eggs laid/female	39	36	9	9	57
Per cent egg hatch	80	65	64	0	94
Per cent females inseminated*	75	69	20	—	—

* By examination of spermathecae.

the basis for comparison. Use of the technique of repetitive matings by single males, with consequent depletion of semen, demonstrated that the "mated effect" was not a result of the physical union during copulation (Table I). Here the expression of the mated type of biting and ovipositional behavior patterns by the females declines with the male's ability to transfer semen successfully, but not with his ability to unite with the female. These results indicate the likelihood that the seminal fluid is the effective agent in modifying these behavioral patterns.

Implantation of the individual organs contributing to the seminal fluid into virgin females demonstrated that the presence of an accessory gland lobe, but not a testis, induced a biting pattern with all the characteristics of normally mated females (Table II). The proportion of the virgin female population which had received a testicular implant, and which fed on day 3, did not differ (65%) from that of the sham-operated (saline-injected) virgins (64%). In contrast, females receiving male accessory gland showed significantly less ($p < .01$) avidity on feeding day 3 (24%) than did either testicular-implanted females (65%) or sham-operated virgins (64%).

While these experiments indicated that alteration of the virgin biting pattern

TABLE II

Effect of implanted male organs in modifying feeding behavior of virgin female Aedes aegypti

Implant	No. feeding	Virgin female feeding behavior; day 3.	
		Mean, per cent	(95% Confidence limits)
Accessory Gland	11/46	24*	(12–39)
Testis	31/48	65	(49–78)
Virgin operated control	27/42	64	(47–79)
Mated operated control	11/29	38	(18–67)

* Significantly less ($p = < .01$) than testis or virgin operated control.

TABLE III

Apholate-induced inhibition of ovarian development and the biting behavior of female Aedes aegypti*

Condition		Number	Females feeding, Day 3	
Female	Male		Per cent	(95% Confidence limits)
A.				
Treated-virgin control		132/167	79	(70–86)
Normal-virgin control		69/96	72	(62–81)
Treated × normal		32/48	67	(52–81)
Normal × normal		28/111	25	(17–34)**
B.				
Treated × normal-operated control		13/16	81	(53–96)
Treated × normal + implanted ovary		2/9	22	(02–59)**

* Exposed to 25–30 ppm Apholate as larvae.
** Proportion of females feeding is significantly less ($p = .02$) than other females within the group.

is brought about by male accessory gland material, an additional component of the control mechanism is indicated by the demonstration that the ovaries of the recipient female also participate in the regulation of this activity. As shown in Table IIIA, biting by Apholate-treated females is always of the "virgin" type, even when these females had mated with normal (untreated) males. In these cases, the absence of a functional ovary abolishes the expected action of the male accessory gland. When these treated females received an implanted normal ovary, however, the expected effect of the accessory gland material (or mating) in altering the virgin pattern is restored (Table IIIB).

A second behavioral characteristic of mated females, which contrasts with the behavior of virgin females, is the readiness of the former to deposit newly matured eggs. The thoracic injection of accessory gland material into virgins caused them to oviposit readily on completion of oogenesis (Table IV). In addition, the proportion of the implanted female population ovipositing is similar to that of the normally mated females. Implantation of a testis did not produce a comparable effect. Dissections of both mated and virgin females during the present experiments demonstrated (Table IV) that equal numbers of eggs were produced by the females regardless of the state of insemination.

DISCUSSION

The distinctness of the biting patterns shown by virgin and mated females of *A. aegypti* has been demonstrated by these experiments and verifies the results of Lavoipierre (1958). The mechanism responsible for modifying the virgin pattern into that of the mated pattern is as yet unknown, but these experiments demonstrate that it is triggered by the accessory gland component of the seminal fluid transferred to the female during copulation. Corollary experiments have demonstrated that no chemical stimulus originating in the testis, nor stimuli associated with either physical union during copulation or the transfer of semen through the female's

TABLE IV

Oviposition by virgin female Aedes aegypti receiving implants of testis or accessory gland

	Implant		Sham-operated	
	Testis	Accessory gland	Virgin	Mated
A. Number of eggs/no. of females:				
Eggs laid	711/27	1905/25	542/31	869/12
Mean/ ♀ ♀	26	76	18	72
Eggs retained*	1430/27	292/20	1930/28	143/10
Mean/ ♀ ♀	53	15	69	14
Total eggs formed				
Mean/ ♀ ♀	79	91	87	96
B. Proportion of test population ovipositing*				
Number	10/27	18/21	6/28	8/10
Per cent	37	86	21	80
(Range)	(13–47)	(75–100)	(0–46)	(75–100)

* Determined by dissection of ovaries: oviposition was considered to have occurred if ovaries contained only a few (<20) mature eggs. Differences in numbers of females due to deaths and escapes.

reproductive tract are effective in inducing this response. When matings are conducted between individual virgin females and sequentially mated ("depleted") males, the results indicate that the male's ability to transfer semen falls off rapidly after 4 to 6 matings. Virgin females allowed to copulate with these males continued to show a "virgin" biting pattern despite the fact that the pair remained *in copulo* for a normal length of time. The ineffectiveness of the physical union *per se* in triggering the mated response is thus demonstrated.

In addition, the thoracic location of the implanted accessory gland rules out possible stimuli associated with sperm transfer within the female's reproductive tract, as occurs in *Cimex lectularis* (Davis, 1965), as an effective stimulus in the alteration of this behavior pattern. The location of the effective implant does, however, point to some diffusible chemical as responsible for the observed changes.

In some insects oogenesis is initiated by the mating act (Davis, 1965; Highnam, 1962; Roth and Stay, 1961), so that changes in feeding behavior might be expected to reflect the new requirements of the activated ovaries. Such a correlation between the state of oocyte development and the activity of the corpus allatum and the preferential ingestion of either protein or carbohydrate seems to occur in *Calliphora erythrocephala* (Strangways-Dixon, 1961) and in *Phormia regina* (Dethier, 1961). Such is not the case, however, in mosquitoes where oogenesis is usually initiated by the ingestion of a blood meal and is independent of whether the female has been inseminated or not (Gillett, 1958; Larsen, 1958; Larsen and Bodenstein, 1959). Neither the pronounced cyclic nature of the mated feeding pattern, nor the difference between mated and virgin female biting patterns, is therefore a consequence of an activated ovary *per se*, or any difference in the numbers of eggs being formed. The suppression of the feeding drive in mated females during the

terminal phases of each gonotrophic cycle thus seems clearly to be a consequence of the presence of the accessory gland material acting in an unknown manner.

Observations of females during the feeding trials indicate that the lowered avidity on day 2, and especially on day 3, is due to the failure of the females to become "activated" (in the sense of Laarman) and to begin flying and seek out the host, rather than to fly and orient to the host and then failing to alight, probe and feed. One effect of the accessory gland material is therefore an apparent increase in the "threshold of activation," and presumably represents a central nervous system phenomenon.

The prominent increase in the feeding rates of the two types of females on day 4 indicates a "release" of the suppressed avidity characteristic of day 3. This release appears to be brought about by the act of oviposition, but other studies indicate this is not the major controlling factor. An increase in feeding rate from 25 to 90% occurs between days 3 and 4 in mated populations. In virgin females the increase is from 70 to 90%, while in mated females which were prevented from ovipositing (unpublished observations), the rate increased from 25 to 70%. A comparison of these feeding rates between virgin and mated females on the one hand, and between ovipositing and non-ovipositing mated females on the other, suggests that the release of suppressed avidity is not due mainly to the oviposition act, but is a consequence of both the completion of oogenesis and the inseminated condition. Thus the release occurring in non-ovipositing mated females amounted to about 70% of that occurring in mated females allowed to oviposit.

Despite the role of male accessory gland material in suppressing the biting drive during the terminal phases of each gonotrophic cycle, further experiments utilizing Apholate-treated females demonstrated that this is not a singular effect, but is expressed only in the presence of a functional ovary. Since the effects of Apholate are undoubtedly broader than just the inhibition of ovarian development, the possibility of "side-effects" and their participation in the abolition of the accessory gland effects exists. This possibility cannot be completely ruled out in the absence of more detailed knowledge of the mode of action of this material. The fact, however, that a normal ovary, when implanted into a treated female, was able to undergo oogenesis, indicates that the nutritional and endocrine environment within the female had not been significantly altered by the treatment. When, in addition, implantation of male accessory glands or mating, re-establishes the feeding pattern seen in non-treated females, the conclusion that the mechanism controlling the biting drive involves both accessory gland material and the ovary seems justified.

Stimulation of oviposition is also a consequence of the presence of accessory gland material. Virgin females bearing an implanted accessory gland oviposited in a manner similar to mated females in regard to the number of eggs laid per female as well as the proportion of the population depositing their eggs (Table IV). These results substantiate the recent findings of Leahy and Craig (1965). They further extend our knowledge of the control of oviposition in *A. aegypti* by demonstrating the ineffectiveness of testicular material in influencing this behavior (Table IV). Matings between virgins and depleted males are also ineffective in increasing the oviposition rate (Table I), thus verifying Gillett's earlier claim (1955) that a component of seminal fluid rather than than physical stimuli induces this behavior.

Little is known of the characteristics of the male accessory gland substance or the factors controlling oviposition in mosquitoes. Lum (1961) had described the gland secretion of several species as a viscous fluid containing granules. Significantly, he points out that within about 24 hours of mating the granular material has dissolved and largely disappeared from the female's reproductive tract. In *Drosophila melanogaster* the analogous paragonial fluid contains a prominent peptide component (Chen and Diem, 1961). Preliminary tests in the present work indicate that the effectiveness of the gland substance in effecting changes in both biting and oviposition behavior is abolished when the material is boiled for 5 minutes prior to injection. Curtin and Jones (1961) have studied the processes of ovulation and oviposition in *A. aegypti*. Their results suggest that the initiation and coordination of these activities are under neural control with cephalic and thoracic regulatory centers. The mechanism coupling the accessory gland material with this neural mechanism remains to be discovered.

Summary

1. When female *Aedes aegypti* were given a daily opportunity to take a blood meal over a 9-day period, the number of mated females feeding on days 3, 6, and 9 was significantly less than the number of virgins feeding. This low period of feeding coincides with the terminal phases of the first, second, and third gonotrophic cycles, respectively.

2. The "mated" feeding pattern could be established in a virgin female by the implantation of a male accessory gland. Copulation without semen transfer or the implantation of a testis was ineffective in inducing the pattern of biting.

3. Functional ovaries in the female receiving the accessory gland implant are required in order for the implant to exert its effect. Implantation of a normal ovary plus an accessory gland into females lacking ovaries produced the mated biting pattern.

4. The material from the male accessory gland also controls oviposition behavior by female mosquitoes. Mated females lay their eggs readily on completion of oogenesis. Virgins mature an equal number of eggs but retain rather than deposit them. Implantation of male accessory gland into virgin females causes them to oviposit on maturation of their eggs.

LITERATURE CITED

CHEN, P. S., AND C. DIEM, 1961. A sex-specific ninhydrin-positive substance found in the paragonia of adult males of *Drosophila melanogaster*. *J. Ins. Physiol.,* **7**: 289–298.

CURTIN, T. J., AND J. C. JONES, 1961. The mechanism of ovulation and oviposition in *Aedes aegypti*. *Ann. Ent. Soc. Amer.,* **54**: 298–313.

DAVIS, N. F., 1965. Studies of the reproductive physiology of Cimicidae (Hemiptera)—III. The seminal stimulus. *J. Ins. Physiol.,* **11**: 1199–1211.

DETHIER, V. G., 1961. Behavioral aspects of protein ingestion by the blowfly *Phormia regina* Meigen. *Biol. Bull.,* **121**: 456–470.

GILLETT, J. D., 1955. Behaviour differences in two strains of *Aedes aegypti*. *Nature,* **176**: 124–125.

GILLETT, J. D., 1958. Induced ovarian development in decapitated mosquitoes by transfusion of haemolymph. *J. Exp. Biol.,* **35**: 684–693.

HAYES, R. O., 1953. Determination of a physiological saline solution for *Aedes aegypti* (L.). *J. Econ. Ent.,* **46**: 624–627.

HIGHNAM, K. C., 1962. Neurosecretory control of ovarian development in *Schistocerca gregaria. Quart. J. Micr. Sci.*, **103**: 57–72.

LAARMAN, J. J., 1958. The host-seeking behaviour of anopheline mosquitoes. *Trop. Geo. Med.*, **10**: 293–305.

LANG, C. A., 1956. The influence of mating on egg production by *Aedes aegypti. Amer. J. Trop. Med. Hyg.*, **5**: 909–914.

LARSEN, J. R., 1958. Hormone-induced ovarian development in mosquitoes. *Science*, **127**: 587–588.

LARSEN, J. R., AND D. BODENSTEIN, 1959. The humoral control of egg maturation in the mosquito. *J. Exp. Zool.*, **140**: 343–381.

LAVOIPIERRE, M. M. J., 1958. Biting behaviour of mated and unmated females of an African strain of *Aedes aegypti. Nature*, **181**: 1781–1782.

LAVOIPIERRE, M. M. J., AND C. L. JUDSON, 1965. A restraining table for implantation and transplantation experiments on mosquitoes. *J. Med. Ent.*, **2**: 85–86.

LEAHY, M. G., AND G. B. CRAIG, 1965. Accessory gland substance as a stimulant for oviposition in *Aedes aegypti* and *A. albopictus. Mosq. News*, **25**: 448–452.

LUM, P. T. M., 1961. The reproductive system of some Florida mosquitoes. II. The male accessory glands and their role. *Ann. Ent. Soc. Amer.*, **54**: 430–433.

MACFIE, J. W., 1915. Observations on the bionomics of *Stegomyia fasciata. Bull. Ent. Res.*, **6**: 205–229.

MACGREGOR, M. E., 1931. The nutrition of adult mosquitoes: preliminary contribution. *Trans. Roy. Soc. Trop. Med. Hyg.*, **24**: 465–472.

ROTH, L. M., AND B. STAY, 1961. Oocyte development in *Diploptera punctata* (Eschscholtz) (Blattaria). *J. Ins. Physiol.*, **7**: 186–202.

STRANGWAYS-DIXON, J., 1961. The relationship between nutrition, hormones and reproduction in the blowfly *Calliphora erythrocephala* (Meig.). II. The effect of removing the ovaries, the corpus allatum and the median neurosecretory cells upon selective feeding, and the demonstration of the corpus allatum cycle. *J. Exp. Biol.*, **38**: 637–646.

WALLIS, R. C., AND C. A. LANG, 1956. Egg formation and oviposition in bloodfed *Aedes aegypti. Mosq. News*, **16**: 283–286.